EVALUATION OF HYPOGLYCEMIC ACTIVITY OF BARRINGTONIA ACUTANGULA FRUIT EXTRACTS IN STREPTOZOTOCIN INDUCED HYPERGLYCEMIC WISTAR RATS

Khatib, N. A.1 AND Patil, P. A.2.

1Department of Pharmacology, K. L. E. University's College of Pharmacy Belgaum 590 010, Karnataka; 2Research foundation, K. L. E. University, Belgaum 590 010, Karnataka.

E.mail: khatibnayeem@hotmail.com

Received: February 12, 2011; Accepted: March 5, 2011

Abstract: The objective of the study is to evaluate the hypoglycemic activity of Barringtonia acutangula (BA) fruit extracts i.e. aqueous, methanol and chloroform in streptozotocin (STZ) 50mg /kg induced hyperglycemic Wistar rats. In addition, the effect of all the three extracts of BA fruit on oral glucose tolerance in glucose loaded normal rats was also determined. Aqueous extract (400 mg/kg) treatment in STZ induced hyperglycemic rats showed significant (P<0.001) decrease in fasting blood glucose levels (BGL) both in acute and chronic study. The activity of the extract was comparable to the standard drug glibenclamide (0.9 mg/kg). Acute administration of aqueous extract (400mg/kg) markedly improved oral glucose tolerance in glucose loaded normal rats, indicating its antihyperglycemic activity. The results of present study indicates that methanol and chloroform extracts do not showed significant hypoglycemic activity whereas aqueous extract of BA fruit possess significant hypoglycemic potential in STZ induced hyperglycemic rats.

Key words: Hyperglycemia, Barringtonia acutangula fruit

INTRODUCTION

Diabetes mellitus is a syndrome characterized by disturbed metabolism of carbohydrate, fat and proteins resulting in high blood glucose level due to lack or ineffective insulin action [1]. The rapidly increasing incidence of diabetes mellitus is becoming a serious threat to human health throughout the world [2]. The oral hypoglycemic agents and insulin preparations are the currently available for the treatment of diabetes mellitus are not free from undesirable side effects [3]. The management of diabetes mellitus thus is global challenge that demands for alternative therapy. The need of the hour, therefore, is to develop indigenous safe and effective herbal formulations free from undesirable effects and cost effective too.

Plant products are well known to mankind since time immemorial to treat a number of ailments by virtue of their contents. The phytoconstituents present in the herbal plants such as alkaloids, terpinoids, flavonoids, phenolics and some other chemical constituents have shown to possess antidiabetic potential [2,4].

Barringtonia acutangula (BA): It is a small to medium sized evergreen tree, native to coastal wetlands in South Asia and Northan Australia. In India it is found in deciduous and evergreen forests along the banks of rivers and streams [5,6]. Different parts, such as leaves, fruit, roots and axillary bud have been used traditionally to treat pains in body, eye ailments, abdominal disturbances, blood impurities, cold asthma, diseases of liver, spleen and
Materials and Methods

Plant materials: The fresh fruit of BA were collected from Yallapur, Uttarakannada district of India, identified and authenticated by Dr. Harsha Hedge taxonomist RMRC (ICMR) Belgaum and a herbarium specimen was deposited with voucher No. RMRC 499. The fresh fruits of the plants were shade dried and reduce to powder.

Preparation of various extracts: The aqueous extract was prepared by macerating 100 gm of powder with chloroform water IP for seven days with occasional shaking at room temperature, filtered and concentrated on rotary evaporator and dried in desicator over sodium sulphite. The yield obtained was 19.68% w/w. Methanol and chloroform extracts were prepared with 100 gm of powder in soxhlet extractor and extracted first with chloroform (40-60 °C) and then with methanol 95% v/v at 60 °C. Appearance of colourless solvent in the siphen tube was considered for termination of extraction process. The yield obtained was 1.6% and 12.08% respectively. All the extracts were stored in refrigerator at 4 °C until further use for experimental study.

Phytochemical screening: A preliminary phytochemical analysis was carried out of all the three extracts (aqueous, methanol and Chloroform) employing the standard phytochemical procedures to reveal the presence of various phytoconstituents.

Animals: Healthy Wistar rats (180-200g) of either sex, obtained from Venkatesh enterprises Bangalore, were used. They were maintained on standard animal pellet diet (Amrut animal feed, Sangli, Maharashtra) and water ad libitum. The present study was dully approved by IAEC bearing Reg. NO 627/02/a/CPCSEA JN Medical College Belgaum.

Acute oral toxicity study: Acute oral toxicity study was carried out by using Wistar rats by “fixed dose” method of OECD guideline NO. 420 and a starting dose of 2000 mg/kg body weight was adopted. There were no toxic effects or mortality observed up to 14th days with all the three extracts.

Evaluation of hypoglycemic activity: BA fruit extracts i.e. aqueous, methanol and chloroform were screened to find out hypoglycemic effect by OGTT method and in STZ induced hyperglycemic rats (acute and chronic model).

i) Oral glucose tolerance test (OGTT): The effect of all three extracts were studied in glucose loaded (3g/kg) normal rats. There were total eight groups consisting each of six normal rats. Group I served as control rats given normal saline (2ml/kg p.o). Group II served as standard group treated with glibenclamide (0.9mg/kg p.o), while groups (III to V) served as test groups treated at a dose 200mg/kg, where as groups (VI to VIII) treated at a dose of 400mg/kg i.e aqueous, methanol and chloroform extracts respectively.

After fasting for 12-16 hrs all animals in each groups were orally administered vehicle, glibenclamide or the different extracts 30 min prior to oral glucose load (3g/kg) respectively. The blood glucose levels were estimated from each group before (0 min) extracts administered and at 30, 60, 90, 120 and 180 min after glucose challenge.

Animal groupings:

Group I: Control (normal saline 2ml/kg)
Group II: hyperglycemic+ glibenclamide (0.9mg/kg) treated
Group III: Test 1 (aqueous extract (200mg/kg)+ glucose 3g/kg)
Group IV: Test 2.(methanol extract (200mg/kg) + glucose 3g/kg)
Group V: Test 3 (chloroform extract (200mg/kg) + glucose 3g/kg)
Group VI: Test 1(aqueous extract (400mg/kg) + glucose 3g/kg)
Group VII: Test 2 (methanol extract (400mg/kg) + glucose 3g/kg)
Group VIII: Test 3 (chloroform extract (400mg/kg) + glucose 3g/kg).

ii) STZ induced hyperglycemic animals: At the
end of one-week acclimatization period, freshly prepared STZ at a dose of 50 mg/kg was injected intraperitoneally as a single dose. Blood samples were obtained from rat tail vein on 3rd and 7th day and blood glucose was estimated by using glucometer. Rats with blood glucose levels of 200 mg/dl or more on both days were categorized as a hyperglycemic.

**Hypoglycemic test:**

**a) Acute study design:** Animals are divided into eight groups with six in each.

- Group I: Hyperglycemic (STZ induced 50mg/kg)
- Group II: Hyperglycemic + glibenclamide (0.9mg/kg)
- Group III: Hyperglycemic + aqueous extract (200mg/kg)
- Group IV: Hyperglycemic + methanol extract (200mg/kg)
- Group V: Hyperglycemic + chloroform extract (200mg/kg)
- Group VI: Hyperglycemic + aqueous extract (400mg/kg)
- Group VII: Hyperglycemic + methanol extract (400mg/kg)
- Group VIII: Hyperglycemic + chloroform extract (400mg/kg)

All STZ induced hyperglycemic rats were fasted for 12-16 hours before they were tested for the blood glucose level. A BGL was recorded on day of experiment prior to the extract administration. Later the animals in each group were orally administered with vehicle, glibenclamide (0.9 mg/kg) or the different extracts (200 & 400mg/kg p.o single dose).

The blood glucose levels were measured at 30, 60, 90, 120 and 180 minutes by using the glucometer.

**b) Chronic study design:**

BA fruit extract showing significant hypoglycemic activity in acute study was only selected for chronic study i.e. for 30 days. The same animals from the acute study were continued for chronic study and treatment given was as follows:

- Group I: Normal euglycemic (normal saline 2 ml/kg)
- Group II: STZ induced hyperglycemic rats. (normal saline 2ml/kg)
- Group III: Hyperglycemic + glibenclamide (900µg/kg p.o single dose)
- Group IV: Hyperglycemic + aqueous extract treated (400 mg/kg p.o twice daily)

**Drugs and chemicals:** Drugs and chemical used includes streptozotocin (Himedia, Mumbai), glibenclamide (Inga Laboratories Mumbai), Glucometer and glucostrips (Sugar check, Wokhardt, Mumbai).

**Statistical analysis:** Data was expressed as Mean ± SEM and was analyzed by two way ANOVA followed by Bonferroni post tests. P<0.05 was considered statistically significant.

**RESULTS**

**Acute toxicity:** All three extracts at a dose 2000 mg/kg body wt. showed no toxic effects or mortality up to 14 days. The LD<sub>50</sub> cut off value of the extracts was 2000 mg/kg body wt.

**Selection of the dose** The LD<sub>50</sub> cut off value was found to be 2000 mg/kg body wt. For the assessment of hypoglycemic activity two doses were selected i.e. first dose 1/10th of the LD<sub>50</sub> cut off value and second dose twice that of one tenth dose i.e. (200 and 400mg/kg respectively).

**Phytochemical screening:** The results of the phytochemical screening of BA fruit extracts showed the presence of carbohydrates, proteins, alkaloids, tannins, saponins, oxalic acid and malic acid in aqueous extract. Carbohydrates and saponins in methanol extract and carbohydrate in chloroform extract.

**Oral glucose tolerance test:** At a dose of 200 mg/kg body wt of aqueous, methanol and chloroform extracts exhibited insignificant fall in a BGL when compared with control group (Fig.1). Acute administration of aqueous extract at dose 400 mg/kg in normal rats showed significant improvement in oral glucose tolerance following oral glucose load as shown (Fig. 2) when compared with control group. No significant fall in BGL observed in methanol and chloroform extracts treated groups.

**Acute hypoglycemic activity:** At a dose 200mg/kg aqueous, methanol and chloroform extracts showed insignificant decreased in BGL in STZ induced hyperglycemic rats when compared with untreated hyperglycemic rats (Table 1). 400 mg/kg dose of aqueous extract showed significantly
Fig. 1: Fasting blood glucose levels of extracts treated rats in OGTT at dose 200mg/kg body wt.

Fig. 2: Fasting blood glucose levels of extracts treated rats in OGTT at dose 400mg/kg body wt.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fasting blood glucose concentration (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Group I</td>
<td>294.00±4.99</td>
</tr>
<tr>
<td>Group II</td>
<td>307.20±4.99</td>
</tr>
<tr>
<td>Group III</td>
<td>302.30±5.55</td>
</tr>
<tr>
<td>Group IV</td>
<td>290.30±4.08</td>
</tr>
<tr>
<td>Group V</td>
<td>302.20±6.11</td>
</tr>
<tr>
<td>Group VI</td>
<td>296.30±3.12</td>
</tr>
<tr>
<td>Group VII</td>
<td>310.80±9.45</td>
</tr>
<tr>
<td>Group VIII</td>
<td>328.80±7.77</td>
</tr>
</tbody>
</table>

Table 1: Effect of acute treatment of various extract of BA fruit at dose 200 & 400 mg/kg body wt. on STZ induced hyperglycemic rats. *P<0.05 when compared with group I. **P<0.01 when compared with Group I. ***P<0.001 when compared with group I.
(P<0.001) decreased the BGL between 30 to 120 minutes. Whereas no significant changes were observed in methanol and chloroform extract treated groups when compared with untreated hyperglycemic rats (Table.1). As aqueous extract at dose 400 mg/kg body wt. significantly decreased BGL with duration of action between 30 to 120 minute, this dose was selected for chronic hypoglycemic activity and was administered twice daily for 30 days.

**Chronic hypoglycemic activity:** Administration of BA aqueous fruit extract at the dose of 400 mg/kg twice daily in STZ induced hyperglycemic rats for 30 days significantly (P<0.001) decreased BGL when compared with untreated hyperglycemic rats.

**Pancreatic histology:** The histopathological studies of pancreas revealed severe congestion with severe decrease in number of islets of Langherhans and beta cells with fibrosis and inflammatory cell infiltration into the islets of Langherhans in STZ induced hyperglycemic rats. While, the aqueous extract of BA whole fruit at a dose of 400 mg/kg showed moderate congestion with moderate decrease in number of islets of Langherhans and beta cell and mild lymphocytic infiltration indicating some amount of recovery. Also the glibenclamide treatment showed moderate congestion with moderate decrease in number of islets of Langherhans and beta cells and mild lymphocytic infiltration (figures are not included).

**DISCUSSION**

The present study was planned to evaluate aqueous, methanol and chloroform extracts of BA fruit on the hypoglycemic effect in OGGT and streptozotocin induced hyperglycemic rats. The acute administration of aqueous extract at 400 mg/kg significantly improved oral glucose tolerance in glucose loaded normal rats indicating its antihyperglycemic activity, acute and chronic study in STZ induced hyperglycemic rats also showed significant antihyperglycemic action. Where as no such effect were seen with methanol and chloroform extracts.

Varios phytochemical constituennts such as polysaccharides [11], flavonoids [12], terpinoids, tannins [13], saponins [14] and alkaloids [15] have shown hypoglycemic activity in animal studies [11-16]. The preliminary phytochemical study of BA fruit extract showed the presence of alkaloids, tannins, saponins and polysaccharides. The hypoglycemic activity of BA fruit could be attributed to the presence of these phytoconstituents. Sulfonylureas, such as glibenclamide known to cause hypoglycemia by stimulating insulin secretion from pancreas [17]. The result obtained from glibenclamide treatment in the present study showed similar significant (P<0.001) hypoglycemic effect. The chronic treatment with aqueous extract of BA fruit for 30 days showed significant (P<0.001) reduction in BGL in STZ induced hyperglycemic rats when compared with untreated hyperglycemic rats.

The histological studies demonstrated severe changes in the pancreas of STZ induced hyperglycemic rats [18,19], whereas BA fruit aqueous extract treatment shows significant recovery when compared with untreated hyperglycemic rats, this could signify the regeneration of beta cells of islets of Langherhans, glibenclamide treatment also showed similar result as reported in literature [17].

The exact mechanism of action of hypoglycemia seen with the BA fruit extract can not be explained on the present results. However it could be proposed that the various phytoconstituents of BA fruit extract and the regenerating property on pancreas be responsible for the hypoglycemic activity. However, further studies are required to establish the exact mechanism of action of the same.

**CONCLUSION**

Aqueous extract of BA fruit at dose of 400 mg/kg twice daily showed significant hypoglycemic activity in STZ induced hyperglycemic rats. Further study is needed to find out the exact mechanism and the phytoconstituents responsible for observed effect.
ACKNOWLEDGEMENTS

The authors are thankful to the Principal Dr. F. V. Manvi and Vice Principal Prof. A.D. Taranalli, KLE College of Pharmacy, Belgaum, Karnataka, India, for the support and constant encouragement.

Abbreviations used: BA = Barringtonia acutangula; STZ = streptozotocin; BGL = blood glucose levels

REFERENCES